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Effects of Salinity Stress on Proline, Phenolic Compounds and Activity of Antioxidant Enzymes in *Dracocephalummoldavica L*

Salehe Naderi[1,*], Mojtaba Seraji[1] and Baratali Fakheri[2]

[1]Department of Plant Breeding, University of Zabol, Iran.

[2]Department of Plant breeding and biotechnology, Agriculture Faculty, University of Zabol, Iran.

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ABSTRACT

Salinity is one of the increasing problems in the world including a wide area of our country. In response to salinity, multiple morphological, physiological and biochemical changes can be created in plants. In this study, Seedlings of *Dracocephalum moldavica* were subjected to 0, 20, 50, 70 and 100 mM NaCl. Changes in morphological traits, proline content, total phenol and activity of antioxidant enzymes such as peroxidase (POX) and polyphenol oxidase (PPO) in shoot were evaluated. As a result, POX and PPO activities in shoot significantly increased while shoot dry weight decreased progressively. The total amount of the phenolic and free proline also increased after the NaCl treatment. The increase was dose-dependent. The highest amount of free proline achived at 100 mM NaCl which was about 3-fold higher than control in shoot, respectively. higher than control. Our results support the idea that the activities of POX and PPO and accumulation of total phenol and proline is associated with salt tolerance as oxidative responses.

Introduction

Dracocephalum moldavica L. is an annual aromatic resistant plant with green leaves and branches belonging to the *Lamiaceae* family. The origin of the plant is reported to be south of Siberia and Himalayan hillsides. Its compounds are sedative and used as appetizer. Its extract has antibacterial effects and is used as a carminative, for stomachache and also in food and beverage industry, cosmetics and hygienic industry (Said-Al Ahl and Abdou, 2010). oil salinity is one of the major abiotic stresses that adversely affect plant productivity and quality (Zhu, 2001). It was estimated that up to 20% of irrigated lands in the world are affected by different levels of salinity and sodium content (Mostafazadeh-Fard et al., 2007). Many crop species are sensitive to high concentration of salt and negative impacts on agricultural production (Zorb et al., 2004). Salinity stress limits plant growth by adversely affecting various physiological and biochemical processes like photosynthesis, antioxidant phenomena, nitrogen metabolism, ion homeostasis (Misra et al., 2006; Ashraf, 2004), proline metabolism and osmolytes accumulation (Misra and Gupta, 2005). Salt tolerant plants can minimize the detrimental effects of high salinities by producing a series of anatomical, morphological and physiological adaptations (Poljakoff- Mayber, 1988). Proline accumulation is one of the most frequently reported modifications induced by salinity and water deficit in plants (Frechilla et al., 2001; Girija et al., 2002), and it is often considered to be involved in stress resistance mechanisms. Phenolic compounds are a large group of secondary metabolites, which can play a role in virtually any interaction a plant can have with its environment (Waterman and Mole, 1994). These compounds have been implicated to stress resistance against biotic and abiotic factors (Bergmann et al., 1994; Cohen et al., 1994; Watermann and Mole, 1994). Oxidative mechanisms in plants include the production of ROS (superoxide radicals (O2.-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH)). Under physiological steady-state conditions, there is a balance between the production and scavenging of ROS (Skopelitis et al., 2006). However, this homeostasis can be disturbed by a number of adverse environmental factors. Plants protect themselves from oxidative damage due to ROS through both enzymatic and nonenzymatic defense mechanisms (Ardıc et al., 2009). Enzymatic ROS scavenging mechanisms in plants include production of superoxide dismutase (SOD), ascorbate peroxidase (APX), Polyphenol oxidase (PPO), catalase (CAT) and glutathione reductase (GR). In this study effect of salinity on morphological traits, amount of proline and phenolic compounds and activity of antioxidant enzymes (POX and PPO) in *Dracocephalum moldavica* L.. was investigated.

Materials and Methods

Plant material and treatment

Dracocephalum moldavica seeds were surface-sterilized for 5 min in sodium hypochlorite solution (0.5%) and then were rinsed with distilled water. Then fifteen seeds were sowed in a plastic pot (20 × 25) contained nonsaline sandy loam soil. Pots were transferred to green house under conditions of 26/18°C day/night temperature and natural light. The pots were irrigated by distilled water. Treatments supplied in five NaCl levels (0, 20,50, 70 and 100 mM) . Treatment was applied to each pot when second leaf was completely expanded and plants were harvested after thirty days.

Morphological traits

Morphological parameters like shoot height was measured in fresh samples. Plants were weighed individually for their fresh weight and then kept for 72 h in oven at 70°C. Finally dry weight was determined by weighing the dried shoots.

Determination of free proline

Proline content was determined based on the method of Bates et al. 0.5 gr plant tissue (shoot) was homogenized with 10 ml of 3% aqueous sulfosalicylic acid and centrifuged for 10 min, 2ml of supernatant were mixed with 2ml of glacial acetic acid and

Tele:

E-mail addresses: salehe.naderi@gmail.com

2ml of acid ninhydrin for 1 h at 100 ◦C. After cooling the reaction mixture, 4 mL of toluene was added and then transferred to a separating funnel. After thorough mixing, the chromophore containing toluene was separated and absorbance read at 520 nm in spectrophotometer against toluene blank. Concentration of proline was estimated by referring to a standard curve of proline.

Total phenols content

Total phenolic content was determined with Folin-Ciocalteu reagent according to the method of Soland and Laima using gallic acid as a standard phenolic compound. In brief, 1 g of shoot samples were placed in an eppendorf tube, with 1 ml of methanol (80%), grinded at 4°C and centrifuged at 14000 × g for 15 min. The extract was mixed with 0.5 ml of Folin-Ciocalteu reagent (diluted 1:1 with water) then 1 ml of a 5% sodium carbonate solution was also added. After 30 min, absorbance was measured at 725 nm.

Ntioxidant enzyme activities

Shoot tissues (1 gr fw) were homogenized in 3 ml 50 mM potassium phosphate buffer, pH 7.0, including 1 mM EDTA, 1 mM PMSF and 1% PVP. The homogenate was centrifuged at 15000 rpm, at 4 ◦C for 20 min.The reactive solution contained 50mM potassium phosphate buffer (pH 7.0), 0.5mM ascorbate, 0.1mM H_2O_2 and 50µL of enzyme extracts. The absorbance was measured at 290nm. Activity was calculated using the extinction coefficient (2.8mM−1 cm−1). One unit of POX was defined as the amount of degrading 1µmol of ascorbate min−1 mg protein−1 under the assay conditions. For PPO measurement the reaction solution contained 0.2 M tris buffer (pH 7.6), 0.02 M pyrogallol and 100 µL of the enzyme extract. Changes in the absorbance of the solution at 420 nm was monitored for 1 min. Activity of PPO was expressed as the amount of purpurogallin formed assuming an extinction coefficient of 2.47 mM−1 cm−1.

Statistical analysis

The experiment was as factorial based on a randomized complete block design with three replications. Data were analyzed using SAS. Means were compared by Duncan Multiple Rang Test (P≤ 0.05).

Results and Discussion

Growth inhibition is a common response to salinity and plant growth is one of the most important agricultural indices of salt stress tolerance as indicated by different studies (Parida and Das 2005). NaCl treatment decreased the shoot length, shoot fresh and dry weights to a large extent (Table 1). Several studies have reported the reduction of shoot length stimulated by salinity (Keutgen and Pawelzik, 2008; Kasukabe et al., 2006; Ibrahim et al., 2007). Results showed that number of leaves significantly (P≤ 0.05) decreased in all levels of salinity compared to the control plants (Table 1). Number of leaves in 100 mM NaCl-treated plants was 69% less than control plants. Reduction in number of leaves per plant is a common phenomenon under salinity stress in various plant species (Zhu et al., 2001). This may be due to the reduction in turgor potential which is necessary for cell elongation (Iqbal and Ashraf, 2005). Similarly, Hassine and Lutts (2010) and Albacete et al. (2008) observed that salinity decreaed the number of leaves in *Zea mays* and *Solanum tubersum*, respectively.

Table 2 shows NaCl induced proline accumulation in shoot. It has been revealed that the proline content substantially increased with increasing NaCl concentrations. The highest concentration of NaCl (100 mM) caused approximately 72% and 89% increase of proline content in shoot, respectively. This is consistent with the reports that NaCl triggered the accumulation of free proline in shoot of *Carthamus tinctorius* and *Pisum sativum* (Hoseini et al., 2010; Najafi et al., 2006). It is generally assumed that proline is acting as a compatible solute in osmotic adjustment (Larher et al., 1993). It may act as an enzyme protectant, stabilize membranes and cellular structures during hostile conditions and detoxify free radicals. Higher level of proline content in stem and leaf maybe due to expression of genes encoding enzymes of proline synthesis such as pyrroline-5-carboxylate or decrease in enzymes of proline oxidative such as proline dehydrogenase which is controlled by osmotic and salinity stress (Amini and Ehsanpour, 2005)

The effect of NaCl on total phenole is shown in Table 2. NaCl at 100mM gave the highest content of total phenol which was 3-fold in shoot respectively, higher than control. Phenol accumulation could be a cellular adaptive mechanism for scavenging oxygen free radicals during stress (Mohamed and Aly, 2008). Several studies have reported that total phenol production is stimulated by NaCl (Hanen et al .,2008; Agastian et al., 2000; Muthukumarasamy et al., 2000).

Effect of NaCl on POX activity is shown in Figure 1 . POX activity in the shoot increased significantly with increasing NaCl concentrations compared to the control and the highest POX activity observed at NaCl concentration of 100 mM. Many studies found a positive correlation between salt stress and the POX activity (Jahnke and White, 2003; Takeda, 2000).

The activity of PPO in treated plants progressively increased with increasing concentrations as compared to control (Figure 2).By increasing of salt concentration from 0 to 100 mM, activity of PPO was increased by 51 % and 74% in shoot, respectively. The increased PPO activity with increasing of salinity levels was recorded under various environmental stresses (Venkatesan and hellappan, 1999). In effective removal of the negative effects of reactive oxygen species which are forming under salt stress conditions can take part PPO together with other enzymes (Niknam et al., 2006).

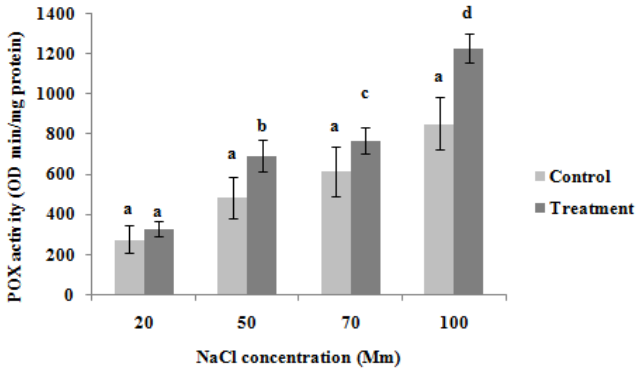

Figure 1. Effect of different NaCl concentrations on POX activity in shoots of *Dracocephalum moldavica* plantlets , Values are mean of three replicates + SD.

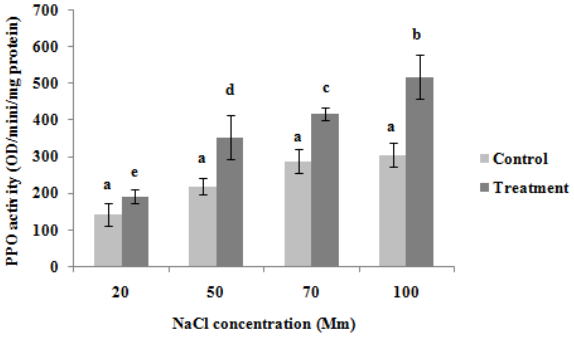

Figure 2. Effect of different NaCl concentrations on PPO activity in in shoots of *Dracocephalum moldavica* plantlets, Values are mean of three replicates + SD

Table 1. The effect salt stress on Dracocephalum moldavica growth parameters (shoot length and fresh and dry weights and number of leaves)

Treatments	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Namber of leaves
0	18.60 a	0.713 a	0.130 a	8.67 a
20	15.55 b	0.656 a	0.114 b	7.00 b
50	13.14 c	0.506 b	0.104 c	5.00 c
70	9.78 d	0.327 c	0.096 d	4.00 cd
100	7.30 e	0.229 d	0.076 e	3.44 d

+ Duncan test (0.05) is to compare mean performances among salinity levels. Different letters represent a significant difference P≤ 0.05 between treatments.

Table 2. Mean of proline and phenolic compounds content (shoot) in Dracocephalum moldavica under different salinity levels

Treatments	Shoot prolin (µmol/g FW)	Shoot total Phenolic compounds (mg/g FW)
0	0.27 a	1.35 a
20	0.37 b	1.55 a
50	0.43 c	2.67 b
70	0.67 c	3.55 c
100	0.86 d	3.87 d

+ Duncan test (0.05) is to compare mean performances among salinity levels. Different letters represent a significant difference P≤ 0.05 between treatments.

In conclusion, the result of this experiment indicates that salinity stress caused a number of morphological and physiological changes in the *Dracocephalum moldavica* plant, including decreased shoot length, fresh and dry weight, number of leaves and increased APX, PPO activities, free proline contant and phenolic compounds. The increased synthesis of proline, total phenolic content and the antioxidant activity in *Dracocephalum moldavica* seedlings exhibited a protective mechanism against the cellular structures from oxidative damage.

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References

Albacete A, Ghanem ME, Martínez-Andujar, M, Acosta M, Sanchez-Bravo J, Martinez V, 2008. Hormonal changes in relation to biomass partitioning and shoot growth impairments in salinized tomato (*Solanum lycopersicum* L.) plants. J Exp Bot. 59: 4119-4131.

Amini F, Ehsanpour AA, 2005. Slouble protein, Carbohydrates and Na+K+ changes in two tomato (*Lycopersicum esculentum* Mill.) cultivars under in vitro salt stress. Am. J. Biochem. Biotechnol. 1: 204-108

Ardıc M, Sekmen AH, Tokur S, 2009. Antioxidant responses of chickpea plants subjected to boron toxicity. Plant Biol 11: 328-228.

Ashraf M, 2004. Some important physiological selection criteria for salt-tolerance in plants. Flora 199: 361 - 376.

Bates LS, Waldern SP, Teare ID, 1973. Rapid determination of free proline for water-stress studies. Plant Soil. 39:205–207.

Bergmann H, Leinhos V, Machelet B, 1994. Increase of stress resistance in crop lants by using phenolic compounds. Acta Hortic. 381: 390–397.

Cohen Y, Treutter D, Feucht W. 2001. Water stress induced changes in phenol composition of Frechilla S, Lasa B, Ibarretxe L et al. Pea responses to saline stress is affected by the source of nitrogen nutrition (ammonium or nitrate). Plant Gr. Regul 35: 171 –179.

Girija C, Smith BN, Swamy PM, 2002. Interactive effects of sodium chloride and calcium chloride on the accumulation of proline and glycinebetaine in peanut (*Arachis hypogaea* L.). Environ. Exper.Bot. 47: 1 –10.

Greenway H, Munns R, 1980. Mechanism of salt tolerance in non halophytes. Annual Review of Plant Physiology. 31: 149-190.

Hanen F, Ksouri R, Megdiche W, Trabelsi N, Boulaaba M, Abdelly C, 2008. Effect of salinity on growth, leaf phenolic content and antioxidant scavenging activity in Cynara cardunculus L. In: biosaline agriculture and high salinity tolerance. Abdelli C, Ozturk M, Ashraf M, Grignon YC, (Eds.). Birkhauser Verlag, Switzerland, pp: 335-343.

Hassine AB, Lutts S, 2010. Differential responses of saltbush *Atriplex halimus* L. exposed to salinity and water stress in relation to senescing hormones abscisic acid and ethylene. J Plant Physiol. 167: 1448- 1456.

Hosseini T, Shekari F, Ghorbanli M, 2010. Effect of salt stress on ion content, proline and antioxidative enzymes of two safflower cultivars (*Carthamus tinctorius* L). J. Food Agric. Environ. 8: 1080-1086.

Ibrahim M, Akhtar J, Younis M, Riaz MA, Anwarul-Haq M, Tahir M, 2007. Selection of cotton (*Gossypium hirsutum* L.) genotypes against NaCl stress. Soil and Environment 26: 59-63.

Iqbal M, Ashraf M, 2005. Changes in growth photosynthetic activity and ionic relations in spring wheat. Plant Growth Regul. 60: 41 -52.

Jahnke LS, White AL, 2003. Long-term hyposaline and hypersaline stresses produce distinct antioxidant responses in the marine algae *Dunaliella tertiolecta*. Plant Physiol. In press. Kar M, Mishra D, 1976. Catalase, peroxidase and polyphenol oxidase activities during rice leaf senescence. Plant Physiol. 57: 315-319.

Kasukabe Y, He LX, Nada K, Misawa S, Ihara I, Tachibana S, 2006. Overexpression of spermidine synthase enhances tolerance to multiple environmental stresses and upregulates the expression of various stress-regulated genes in transgenic *Arabidopsis thaliana*. Plant Cell Physiology. 45:712-22.

Keutgen AJ, Pawelzik E, 2008. Quality and nutritional value of strawberry fruit under long term salt stress. Food Chemistry 107:1413-1420.

Larher F, Report L, Petrivalsky M, Chppart M, 1993. Effectors for the osmoinduced proline respose in higher plants. Plant Physiol. Biochem. 30: 911 -922.

leaves and phloem of *Prunus avium* L. Acta Hortic 381: 494–497. 1994.

Misra N, Gupta AK, 2005. Effect of salt stress on proline metabolism in two high yielding genotypes of green gram. Plant Sci. 169: 331 -339.
Misra N, Gupta AK, Dwivedi UN, 2006. Changes in free amino acids and stress protein synthesis in two genotypes of green gram under salt stress. J Plant Sci. 1: 56-66.
Mohamed AA, Aly AA, 2008. Alternations of some secondary metabolites and enzymes activity by using exogenous antioxidant compound in onion plants grown under seawater salt stress. AmericanEurasian Journal of Scientific Research. 3: 139-146.
Mostafazadeh-fard B, Heidarpour M, Aghakhani QA, Feizi M, 2007. Effects of irrigation water salinity and leaching on soil chemical properties in an arid region. Int. J. Agr. Biol. 3: 166-469.
Muthukumarasamy M, Gupta SD, Pannerselvam R, 2000. Enhancement of peroxidase, polyphenol oxidase and superoxide dismutase activities by triadimefon in NaCl stressed *Raphanus sativus* L. Biol. Plant. 43: 317-320.
Najafi F, Khavari RA, Rastgar JF, Sticklen M, 2006. Physiological changes in Pea (*Pisum sativum* L. cv. Green Arrow) under NaCl salinity. Pak. j. Biol. Sci. 9: 974-978.
Nakano Y, Asada K, 1981. Hydrogen peroxide scavenged by ascorbate-specific peroxidase in spinach chloroplast. Plant & Cell Physiology. 22: 867-880.
Niknam V, Razavi N, Ebrahimzadeh H, Sharifizadeh B, 2006. Effect of NaCl on biomass, protein and proline contents, and antioxidant enzymes in seedlings and calli of two *Trigonella* species. Biol. Plant. 50: 591 - 596.
Parida AK, Das AB, 2005. Salt tolerance and salinity effect on plants: a review. Ecotoxicol. Environ. Saf. 60: 324–349.
Poljakoff-Mayber A, 1988. Ecological-physiological studies on the responses of higher plants to salinity and drought. Sci. Rev. Arid Zone Res. 6: 163-183.
Said-Al Ahl, H.A.H. and M.A.A. Abdou, 2010. Impact of water stress and phosphorus fertilizer on fresh herb and essential oil content of dragonhead. I. A., 23: 403-407.
Skopelitis DS, Paranychianakis NV, Paschalidis KA,. 2006. Abiotic stress generates ROS that signal expression of anionic glutamate dehydrogenases to form glutamate for proline synthesis in tobacco and grapevine. The Plant Cell 18: 2767-2781.
Soland SF, Laima SK, 1999. Phenolics and cold tolerance of *Brassica napus*. Plant Agri. 1: 1 -5. Takeda T, Yoshimura K, Yoshii M, Kanahoshi H, Miyasaka H, Shigeoka S, 2000. Molecular characterization and physiological role of ascorbate peroxidase from halotolerant *Chlamydomonas* sp. W80 strain. Arch. Biochem. Biophy., 376: 82-89.
Venkatesan A, Chellappan KP, 1999. Salinity effect on the activities of certain antioxidant enzymes in *Ipomoea pes-caprae* sweet, a halophyte. Indian J. Plant Physiol. 4: 40-42.
Waterman PG, Mole S. 1998. Analysis of phenolic plant metabolites. Bluckwell Scientific Publications. Oxford. London. Zhu JK, 2001. Plant salt tolerance. Trends Plant Sci. 6: 66-72.
Zorb C, Schamit S, Need A, Karl S, 2004. The biochemical reaction of maize (*Zea mays* L.) to salt stress is characterized by mitigation of symptoms and not by specific adaptation. Plant Sci. 167: 91 -100.